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Report No. IITRI-L6023-10
(Quarterly Status Report)

LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

Contract No. NASr-22

National Aeronautics and
Space Administration
Washington, D.C.

IIT RESEARCH INSTITUTE

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I. INTRODUCTION

Studies on the survival and growth of Staphylococcus aureus in severe environments are in progress. Growth of S. aureus occurred within 3 to 28 days under the following environmental conditions:

- (1) 100% carbon dioxide at 5 mb pressure, 0.92 and 0.94 a_w , 16-hr and 20-hr daily freeze.
- (2) 100% carbon dioxide at 10 mb pressure, 0.92 and 0.94 a_w , 16-hr and 20-hr daily freeze.
- (3) 67% carbon dioxide at 25 mb pressure, 0.92 a_w , 16-hr and 20-hr daily freeze.
- (4) 67% carbon dioxide at 25 mb pressure, 0.82 a_w , 16-hr daily freeze.

Soil ecology experiments studying the growth responses of microorganisms in different soils with a simulated Martian environment (67% carbon dioxide at 15 mb pressure, 16-hr daily freeze) were started. These studies, related to the probability of extraterrestrial contamination, will evaluate the minimum

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moisture and numbers of the following microorganisms required for growth and survival: Bacillus cereus, Lactobacillus plantarum, Pseudomonas aeruginosa, Putrefactive Anaerobe (PA 3679), S. aureus, and Streptomyces albus.

Construction of the Martian chamber was completed. Initial trial runs for leak testing were successful. At the present time preliminary experiments are being conducted in this chamber to study changes in the native microbial flora of a brunizem soil subjected to different moisture concentrations and temperatures.

The following two papers were presented during this report period:

- (1) Probability of Growth (P_G) of Viable Microorganisms in Martian Environments. E. J. Hawrylewicz, C. A. Hagen, Vivian Tolkacz, B. T. Anderson, and Marjorie Ewing. COSPAR, International Space Science Symposium, London, England. July, 1967.
- (2) Ability of Microorganisms to Establish Ecological Niches in Different Soils and Environments. C. A. Hagen, E. J. Hawrylewicz, B. T. Anderson, Vivian Tolkacz, and Marjorie Ewing. Soc. for Industrial Microbiol., London, Ontario, Canada. August, 1967.

II. EXPERIMENTAL PROCEDURES

Stock culture preparations of the microorganisms were described in Reports No. IITRI-L6023-5, -6, and -7. For the soil ecology experiments, the preparation and inoculation of test tubes and the methods for determining water activity (a_w) of the microorganisms were described in Report No. IITRI-L6023-9.

All stock culture cell suspensions were stored at 4°C until used. B. cereus and PA 3679 spore suspensions were heat-shocked at 80°C for 10 min just before use.

The soil ecology experiments started this report period utilized the following environmental conditions:

- (1) Gaseous Composition - 67% carbon dioxide, 30% nitrogen, and 3% argon.
- (2) Atmospheric Pressure - 15 mb.
- (3) Temperature Cycle - 16-hr freeze at -65°C and 8-hr thaw at 25°C.
- (4) Moisture Concentration - maximum to minimum a_w depending on the organism's requirement.
- (5) Soil Type - brunizem, desert, and podzol.
- (6) Organism - B. cereus, L. plantarum, PA 3679, P. aeruginosa, S. aureus, and S. albus.
- (7) Cell Concentration - usually three levels: 10^3 , 10^2 , and 10 cells/g of soil.

The experiments are divided into key, or screening, and testing, or confirmatory types. The key experiments determine the lowest concentration of moisture the smallest number of cells

require for survival in the environment over a seven day test period. The testing experiments consider the two lowest numbers of cells and the minimum a_w for survival and growth in the environment over a 56-day test period.

Bacterial counts are reported as averaged counts of two plates from each of three tubes. Incubation was at 35, 30, or 25°C for 1 to 5 days, depending on the bacterial species.

III. RESULTS AND DISCUSSION

The effect of 100% carbon dioxide at 5 mb pressure on S. aureus is shown in Figure 1. Growth occurred in the tubes containing 0.94 a_w within seven days with a 16-hr daily freeze and within 28 days with a 20-hr daily freeze. Extension of the time frozen delayed growth and has been reported in our previous studies with other environments and organisms. Lowering the moisture concentration to 0.92 a_w resulted in a further delay of growth.

Figure 2 shows the effect of the same atmosphere at 10 mb pressure on S. aureus. Growth occurred between 7 and 28 days at both moisture concentrations, 0.92 and 0.94 a_w , and both freeze cycles.

In both environments there were instances of a large decrease in numbers of viable cells recovered after growth had occurred. The loss in viability does not appear to be solely related to barometric pressure, gaseous composition, moisture concentration, or length of daily freeze. However, in similar environments but with maximum a_w 's viability was not as greatly affected.

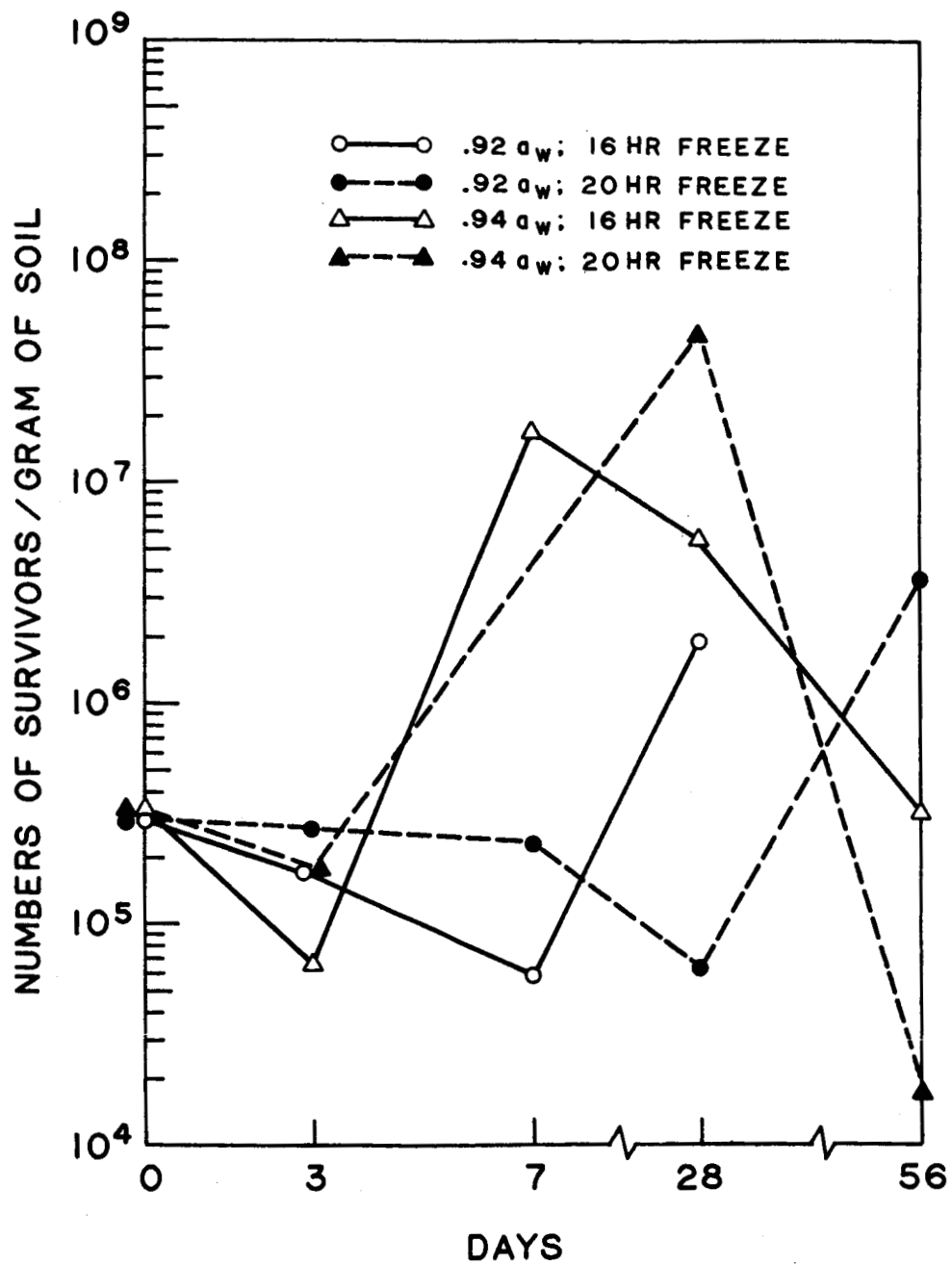


FIG. 1 EFFECT OF 100% CARBON DIOXIDE ATMOSPHERE AT 5mb PRESSURE ON GROWTH AND SURVIVAL OF STAPHYLOCOCCUS AUREUS.

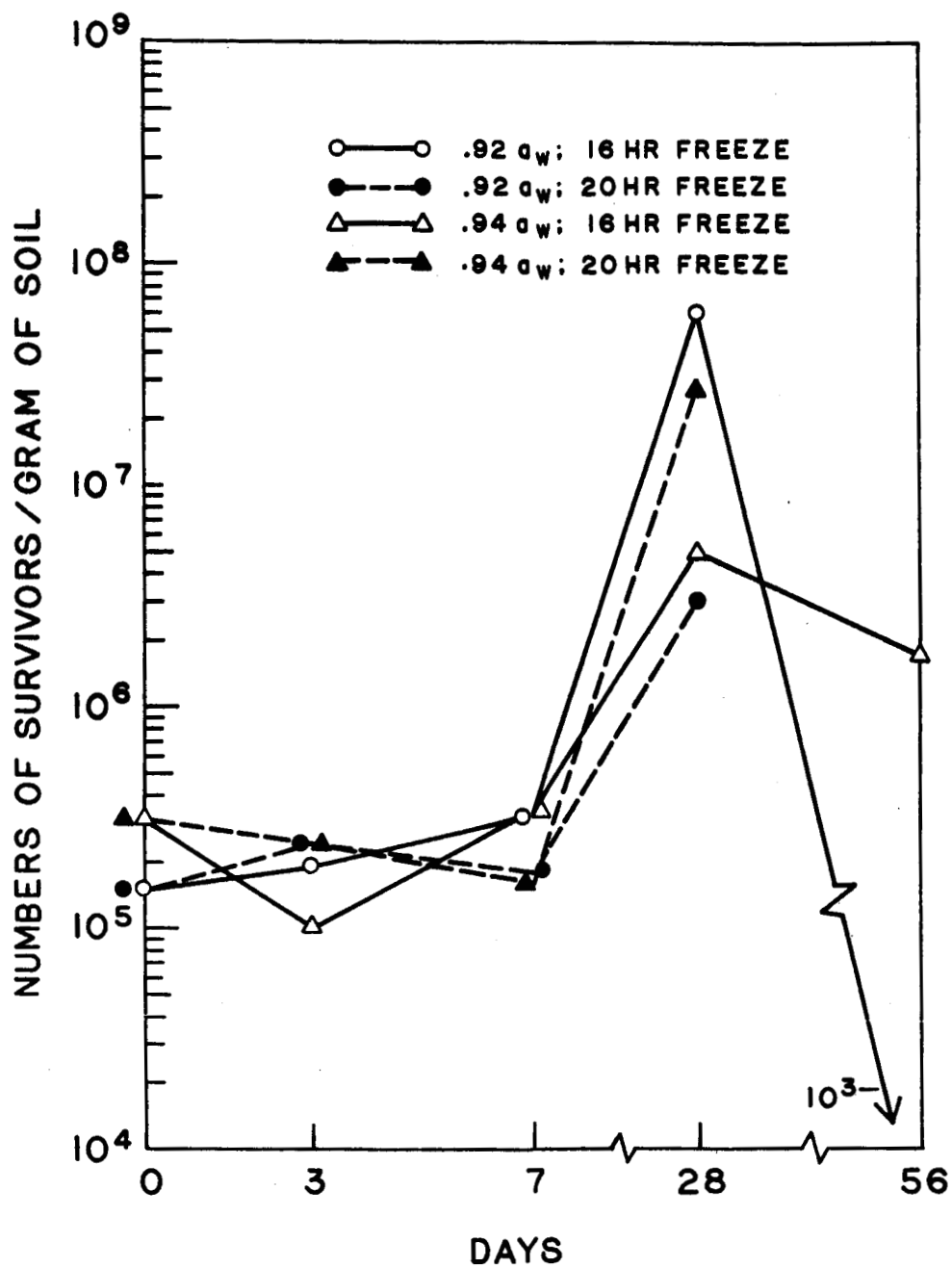


FIG. 2. EFFECT OF 100% CARBON DIOXIDE ATMOSPHERE AT 10mb PRESSURE ON GROWTH AND SURVIVAL OF STAPHYLOCOCCUS AUREUS.

Growth of S. aureus in an environment with 67% carbon dioxide (with 30% nitrogen and 3% argon as the balance) at 25 mb pressure is shown in Figure 3. Growth occurred within seven days with 0.92 a_w and a 16-hr daily freeze, within 28 days with 0.92 a_w and 20-hr daily freeze, and within 28 days with 0.82 a_w and 16-hr daily freeze. Growth had not occurred by 28 days with 0.82 a_w and 20-hr daily freeze. Both the extension of the daily freeze time and the lowering of the a_w resulted in a delayed growth response.

IV. SUMMARY

S. aureus survived and grew in a 100% carbon dioxide atmosphere at 5 and 10 mb pressures with 0.92 and 0.94 a_w 's. Increasing the daily freeze time from 16 to 20-hr delayed the growth response. In either event, growth occurred within 3 to 28 days with maximum populations 2 logs higher than initial populations.

S. aureus survived and grew in a 67% carbon dioxide atmosphere (balance was 30% nitrogen and 3% argon) at 25 mb pressure, 0.92 a_w with either a 16-hr or 20-hr daily freeze. Growth also occurred in a similar environment with 0.82 a_w and a 16-hr daily freeze.

Experiments to determine minimum numbers of different microorganisms required to establish an ecological niche in different soils with a 67% carbon dioxide atmosphere at 15 mb, a 16-hr daily freeze, and minimum a_w were started.

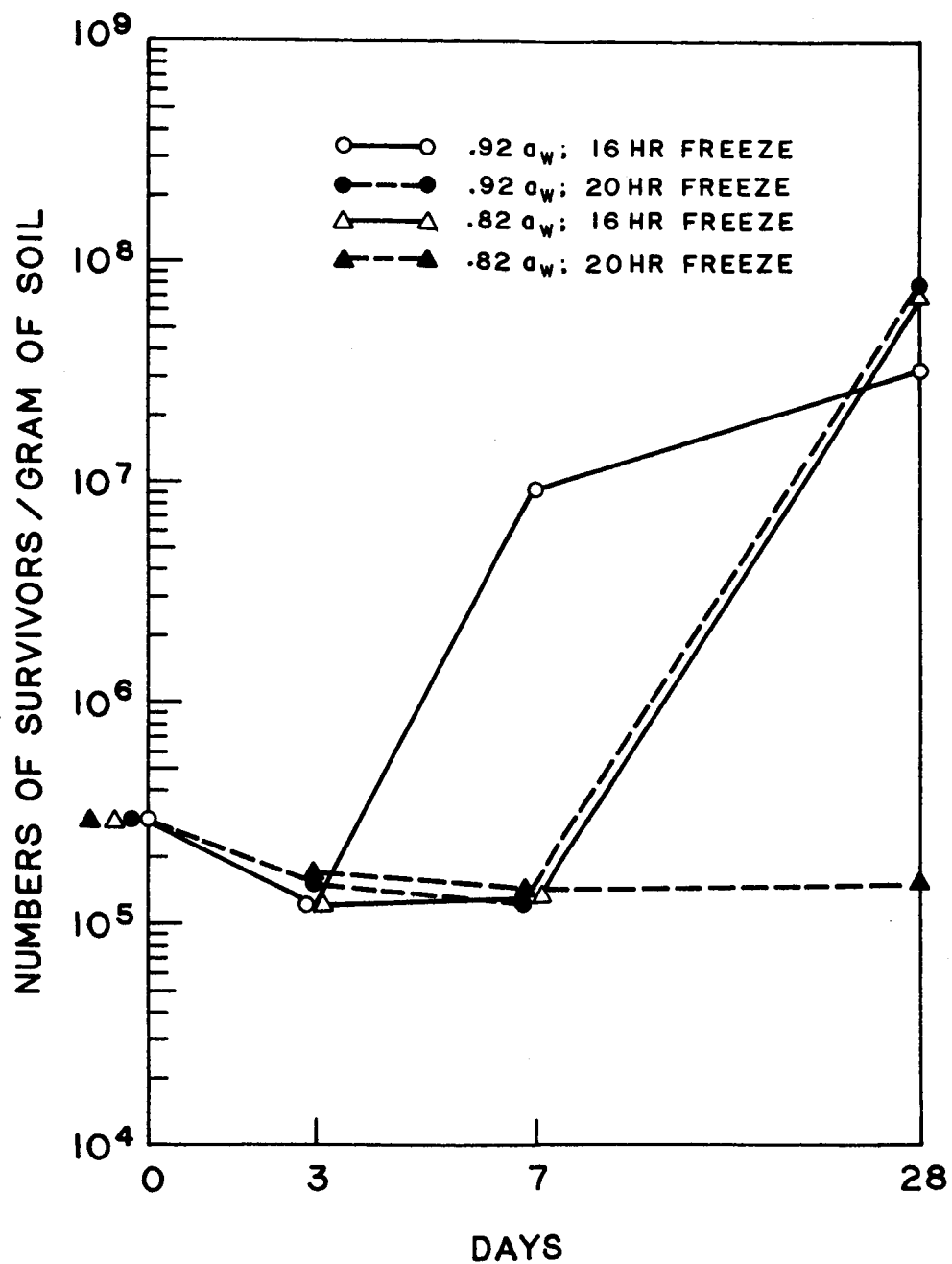


FIG. 3 EFFECT OF 67% CARBON DIOXIDE ATMOSPHERE AT 25mb PRESSURE ON GROWTH AND SURVIVAL OF STAPHYLOCOCCUS AUREUS.

Preliminary experiments studying changes in native flora of a brunizem soil are being performed in the newly constructed Martian chamber.

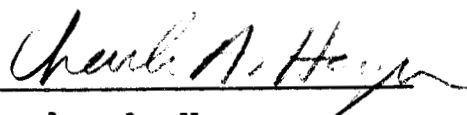
V. PERSONNEL AND RECORDS

The experiments were planned with the counsel of Dr. E. J. Hawrylewicz and the technical assistance of Mr. Bruce Anderson, Miss Marjorie Ewing, and Miss Vivian Tolkacz.

Experimental data are recorded in IITRI Logbooks C17605, C17587, C17849, and C18029.

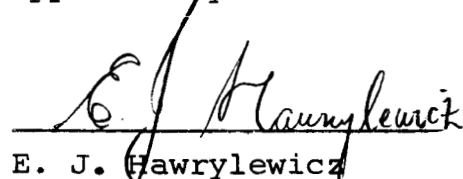
Respectfully submitted,

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